FURTHER INVESTIGATIONS ON THE EMBRYOLOGY OF VIVIPAROUS SEEDS.

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(Plate xv; thirty-eight Text-figures.)

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The embryology of *Avicennia officinalis* was investigated by Treub in 1883. Some years later, Karsten (1891) published an extensive work embracing all known species of mangroves.* Haberlandt (1895) examined the embryos of several genera from the point of view of nutrition. Cooke (1917) amplified Karsten's brief mention of the embryology of *Rhizophora Mangle.* Recently, Carey and Fraser (1932) described the embryology of *Aegiceras majus.*

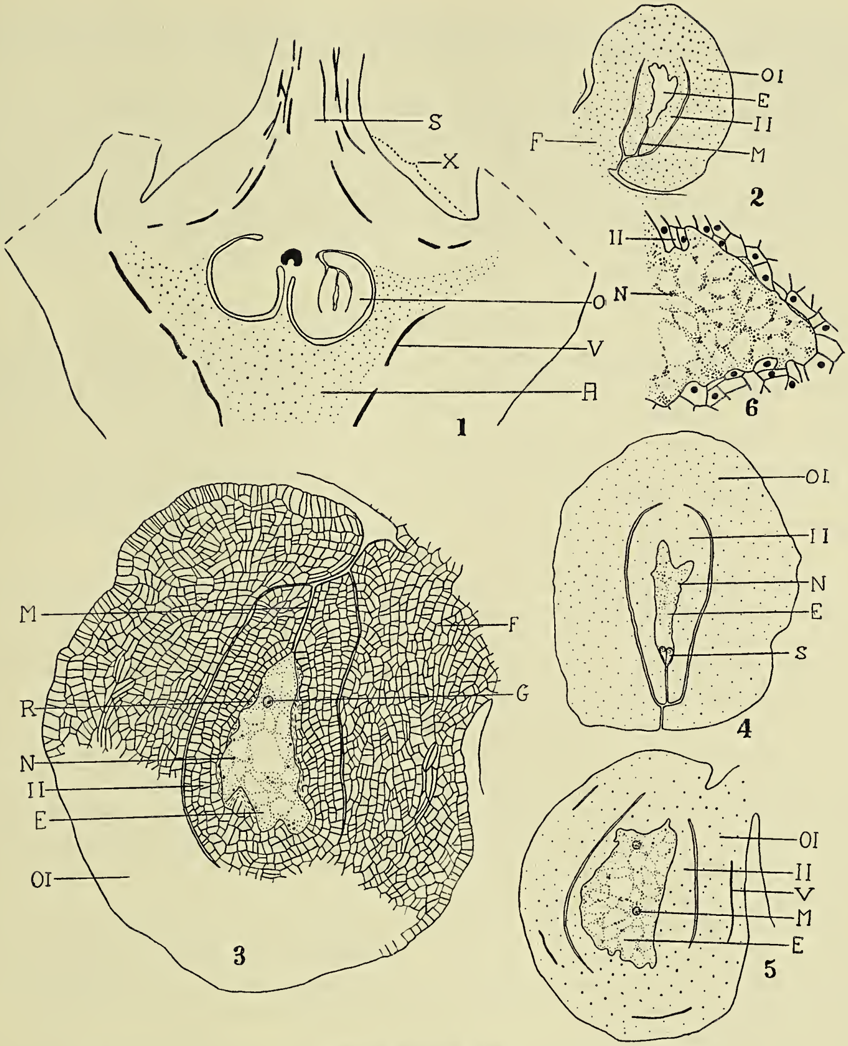
Embryological material of *Rhizophora mucronata* and *Ceriops Candolleana* obtained in the Southern Tropics from the coastal town of Yeppoon, about twenty-five miles from Rockhampton, Queensland, was available to the writer. This material showed that a re-examination of the embryology of these types would prove interesting.

Rhizophora mucronata Lam.

As the general structure of the flower of *Rhizophora mucronata* has been described by systematists (Bentham, Manson Bailey), it will not be included here, but the ovary and receptacle deserve further mention.

The gynoecium.—An examination of the wall of the half inferior ovary shows an interesting arrangement of tissues, where it is fused to the receptacle. The outer walls of the epidermal layer are thickened and covered with a wide cuticle. Beneath this peripheral layer lies a small-celled tissue in which groups of 'stone' cells are freely scattered. Passing inward, there next occurs a wide band of large parenchymatous cells among which branched sclereids are scattered freely. Sclereids of this type are characteristically found in many parts of the plant body among species of this family, as has been noted by Solereder (1908). The parenchymatous cells of this area are filled with a densely granular cytoplasm and those lying on the inner side in close proximity to the vascular strand contain druses of calcium oxalate. Inside this tissue lies an aerenchyma (A in Text-figure 1) which, extending round the loculus and passing up under the other floral parts, eventually merges into the more compact tissue of the ovary wall at the base of the style. Here the cells which are also parenchymatous are arranged in compact vertical rows. The cells are cubical, but gradually become vertically elongated toward the middle and apex of the style. The presence

* Karsten's "Ueber die Mangrove-Vegetation im Malayischen Archipel" is unavailable in Australia. However, through the courtesy of Sir Arthur Hill, Director of Kew Gardens, England, a typewritten copy of pages 11-17 and 31-41 was made available to the writer, who wishes to express her sincerest thanks to Sir Arthur Hill.



Text-figures 1-6.

1.—A diagram of a longitudinal section of the ovary of a flower of *Rhizophora mucronata*. S, style; X, zone which later increases most in size; V, vascular tissue; O, ovules; A, aerenchyma (x 11·5).

2.—An ovule of *Rhizophora mucronata* showing the intrusion of the embryo sac into the inner integument. OI, outer integument; II, inner integument; M, micropyle; F, funicle; E, embryo sac (x 41).

3.—A detailed study of the ovule as shown in Text-figure 2. OI, outer integument; II, inner integument; E, embryo sac; N, deeply staining granules; R, resorbed tissue of the inner integument; F, funicle; M, micropyle; G, egg nucleus (x 112).

4.—A tangential section of an enlarging ovule showing the haustorial action of the embryo sac. OI, outer integument; II, inner integument; E, embryo sac; N, deeply staining granules; S, synergids (x 41).

5.—An enlarging ovule of *Rhizophora mucronata* before fertilization. OI, outer integument; II, inner integument; E, embryo sac; V, vascular tissue; M, endosperm nucleus (x 41).

6.—A detailed study of a portion of the embryo sac shown in Text-figure 5. II, inner integument; N, deeply staining granules (x 208).

of aerenchyma in close association with the ovules not only of this genus but of other truly viviparous plants such as *Bruguiera* and *Ceriops* seems to be significant.

Dividing the cavity of the ovary into two loculi is the placenta. To it are attached four anatropous ovules, each by its own massive funicle. These ovules are joined to the placenta at the stylar end, just above the latter's horseshoe-shaped vascular strand (Text-figure 1). This strand passes through the placenta in a horizontal direction till it joins the conducting tissue running vertically up the ovary wall. The lower part of the narrow placenta is composed of parenchymatous cells, staining deeply with dyes.

Development of Ovule.

In the youngest ovule examined by the writer the embryo sac was clearly differentiated. However, the stages prior to this are fully described by Karsten (1891). The embryo sac lies in a nucellus, which is surrounded by two rather massive integuments. The outer integument is the more massive and towards its periphery a vascular strand is soon initiated, passing three-quarters of the way round the ovule. The narrow micropyle which is directed toward the placenta is not coincident through the two investments (Text-figures 2, 3, M), but takes a sharp turn between the inner and outer integuments.

Before the bud opens the enlargement of the embryo sac results in the complete disappearance of the nucellus. Then, as the flower expands, the ovule increases in size by divisions within the integuments, accompanied by the continued enlargement of the embryo sac, which at this stage attacks the tissue of the inner integument, throwing out haustorial folds (Text-figures 2, 3). The resorption of the inner integument covers a considerable period of time, as the tissue being invaded is constantly dividing. In fact, the ovule increases to approximately four times its size at anthesis before resorption is complete (compare Text-figures 2, 7). This resorption is greatest at the chalazal end of the embryo sac, where the larger haustorial folds enter the integumental tissue (Text-figures 2, 4, 5). The resorption of the cells by one of these folds of the embryo sac is shown in Text-figure 6.

Towards the micropyle the resorption is slower and it is there that the most actively meristematic cells of the inner integument are found. Thus by their division they maintain, for a period, a considerable width of this integument in the micropylar region, and are last to disappear under the haustorial action of the embryo sac (Text-figure 7).

During this development the different staining properties of the inner and outer integuments become very marked. The inner integument stains more lightly than previously with basic dyes such as aniline blue, while the outer integument which has become densely packed with tannin and food material, strongly holds any dye into which it is placed.

In Karsten's account, no description is given of the appearance of the two integuments or of their development as the embryo sac enlarges, although these features might be shown in his text-figures, which were not available to the writer. He simply states that the inner integument undergoes resorption while eventually only a few cells remain at the micropylar end.

Meanwhile, within the embryo sac, the female gametophyte has been differentiated. Karsten records that the first division of the embryo sac nucleus occurs after the resorption of the nucellus. Two pear-shaped synergids lie at the micropylar end of the embryo sac (Text-figure 4, S) close to the egg (Text-figure 3, G). Towards the middle of the embryo sac is the endosperm nucleus (Text-figure 5, M). The antipodal cells, Karsten states, are not visible in the growing embryo sac, and this has been confirmed by the present investigation.

In the embryo sac of the young flower a few granules are scattered evenly throughout the cytoplasm (Text-figures 3, 4, N). These granules, which may be of a nutritive nature, gradually decrease in size as they increase in number with the enlargement of the embryo sac (Text-figure 6, N).

Fertilization.

Actual fertilization has not been observed by the writer, but Text-figure 7 shows what are apparently the remains of pollen tubes passing through the inner integument. This fact was also recorded by Karsten, so fertilization must occur at a stage between those shown in Text-figures 5 and 7.

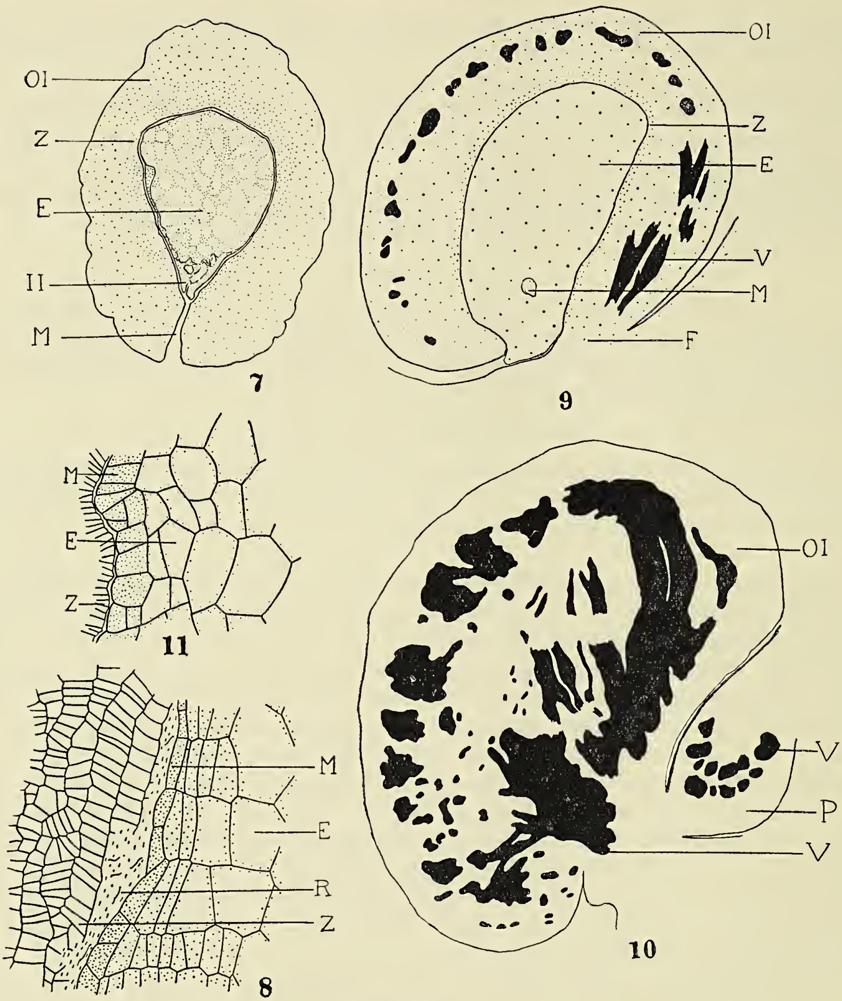
Post-fertilization Development.

All four ovules may develop to fertilization, but after that, one gradually becomes dominant, causing the abortion of the other three. The petals and stamens fall away, leaving the enlarging ovary surrounded by the persistent calyx.

After fertilization the remnants of the inner integument are resorbed and cell wall formation occurs in the embryo sac (Text-figure 8, E). The cells so formed evidently arise first at the periphery and then in radiating series towards the centre, forming an endosperm of large thin-walled cells. The peripheral layer of this endosperm lying next the integument is clearly differentiated from the rest of the endospermic tissue by the densely protoplasmic nature of its cells, as well as by their smaller size (Text-figure 8, M). This layer, which was noted by Haberlandt in the older stages of the seed and compared by him with the 'epithelial' layer of grass embryos, is a meristematic one. It extends right round the endosperm and for a time its cells divide equally, but later the divisions in the micropylar region become the more rapid, resulting in the passage of the endosperm into the loculus. The cells throughout the endosperm are densely packed with food material similar to that which is found in great quantities in the integumental cells.

The endosperm is surrounded by the integument in which differentiation has occurred. Just previous to the formation of the endosperm, indications of two distinct zones become evident (Text-figure 7, Z and OI). These gradually become more distinct as the fertilized ovule enlarges (Text-figure 9, Z and OI). In the larger and outer zone (OI) is an extensive development of vascular tissue, shown in Text-figures 9, 10 (V). These conducting strands lie in a ground tissue of small parenchymatous cells, forming a zone which expands slowly and gives rigidity to the developing seed. Throughout its development it remains free from the invasion by the endosperm.

Inside this zone lies a narrower one (Text-figure 9, Z), extending right round the inner limit of the integument, except in the region of the micropyle. This zone is formed of cells arranged in several rows parallel to the circumference of the endosperm against which they abut. The junction between these two tissues is marked by slight irregularities due to the absorption of the integumental cells. This zone divides radially at a rate rather quicker than that at which the endosperm is resorbing it, so, for a time, it increases in width radially.



Text-figs. 7-11.

7.—A tangential section of an ovule showing the last remnants of the inner integument. OI, outer integument; Z, inner zone of the integument; II, inner integument; E, embryo sac; M, micropyle (× 23).

8.—A portion of a developing seed showing the endosperm. R, resorbed tissue of the outer integument; E, endosperm; Z, inner zone of the outer integument; M, meristematic zone of the endosperm (× 208).

9.—Diagram of a longitudinal section of a developing seed of *Rhizophora mucronata* in which the endosperm has developed for some time. This section passes through the outer edge of the micropyle, but not through the slit in the integument. Z, inner zone of the integument; OI, outer integument; V, vascular supply; E, endosperm; F, edge of the funicle; M, embryo (× 11·5).

10.—A tangential section through the integument of an enlarging seed of *Rhizophora mucronata,* showing the extensive development of vascular tissue. OI, outer integument; P, placenta; V, vascular tissue (× 11·5).

11.—A detailed study of a portion of a seed such as that shown in Text-figure 9. Z, inner zone of the outer integument; M, meristematic layer of the endosperm; E, endosperm (× 208).

Judging by the staining properties of these cells, enzymes do not penetrate more than two or three cell layers of the outer integument. However, the most rapid divisions occur parallel to the long axes of the cells. These take place with such rapidity that the rows of cells, instead of being quite vertical, become slightly folded so that the endosperm which lies in intimate contact seems to penetrate this food-packed area in gentle undulations (Text-figure 11).

One of the striking characteristics of the whole integument is the smallness of its cells, which are only a little larger than those in the ovule of the open flower. The size of the cells, combined with the very abundant food supplies which they hold, may account for the greater resistance of this outer integument to the endosperm.

The enlargement of the integument carries that part of the developing seed opposite the funicle well beyond the micropyle, which then appears to be eccentrically placed (Text-figure 12). However, all the cells in this part of the integument do not divide at an even rate and, by their differential growth, the integument is divided in the micropylar region into two equal lobes. These lobes are separated by a slit (D) running from the outer edge of the integument into the micropyle (M). The base of this slit is oblique, as it is much deeper at the micropylar than at the outer edge.

The micropyle itself, which was originally tubular, is now but a circular aperture at which the edge of the endosperm can be seen (Text-figures 9, 12, M). As the developing ovule enlarges, the micropyle gapes widely (M) and the slit in the integument (D) gradually opens (Text-figures 13, 14), till an almost circular aperture of considerable size is formed, through which the broad face of the endosperm advances. This expansion is purely mechanical, occurring as a result of the force exerted on the outer zone of the integument by the rapid divisions in the inner zone. This view with regard to the opening of the micropyle is contrary to the view expressed by Karsten, who definitely states that it is not due to the growth of the integument but is governed by the pressure of the embryo. "Das wirkliche Auseinanderdrängen der Mikropyle glaube ich in allen Fällen dem Keimlinge selbst zuschreiben zu müssen, soweit es sich nicht um actives Wachsthum des Integumentes handelt."

The embryo, which till now has been ignored in this discussion, is well differentiated before the endosperm emerges from the seed. The youngest embryo in the material at the writer's disposal was found in the endosperm at the micropylar end of a seed 0·4 cm. in length (Text-figure 9, M). It consists of an undifferentiated mass of embryonal tissue (Text-figure 15, E), attached by a massive suspensor (S) to the surrounding nutritive endosperm. This suspensor differs from that of *Rhizophora Mangle*, which Cooke (1907) describes as a linear series of cells. The embryo grows as the endosperm enlarges so that, by the time the seed is one centimetre in length, it can be clearly discerned with the naked eye (Text-figure 16, E). There are two cotyledons, which are fused throughout the greater part of their length, while the free tips are closely appressed to one another.

The endosperm, by the division of the outermost layer of its cells, now emerges through the micropylar opening into the loculus of the ovary. It endeavours to penetrate the ovary wall. However, this action is slow compared with the rate of increase of the endosperm, so as a result this tissue spreads out in the chamber of the loculus and falls back over the integument of the seed. In extreme cases the endosperm seems to cover the integument almost entirely.

Development of the Ovary.

By enzyme action the advancing face of the endosperm invades the ovary wall which has been undergoing change to accommodate the enlarging fertilized ovule. In order to understand the structure of the ovary wall at this stage, it will be necessary to return to the ovary as it is in the flower and trace the changes which occur in its tissues till the time now under discussion.

In the flower, as the ovules enlarge the young ovary expands differentially, the greatest development being in that region of the ovary wall lying between the base of the style and the loculus (X in Text-figure 1). The remainder of the ovary wall just keeps pace with the growth of the contained ovules and, after fertilization, with that of the dominant fertilized ovule, which crushes to one side the second loculus of the ovary in which the other ovules have aborted. During this growth the divisions of the placenta lag behind those of the growing seed (Text-figure 16, P). Thus the placenta, as the seed enlarges, becomes tightly drawn against it, causing a deeper and deeper depression in the integument (Text-figures 13, 14, C, 25, D).

The most remarkable expansion, as mentioned above, is in that part of the ovary wall at the base of the style. In this region of the flower the parenchymatous cells are arranged in more or less vertical rows, among which extend the vascular strands connecting with the apex of the style. As the flower ages and the petals fall, these parenchymatous cells divide with increasing rapidity till this zone becomes an extensive conical mass of tissue (Text-figure 16, X), at whose apex can be seen the two fused styles (Text-figure 16, S) surmounted by the bifid stigma. An examination of the tissues of this cone shows that there is a central area (Text-figure 16, A) cut off from the rest of the wall by vascular strands (V), as in the young flower (Text-figure 1). The cells of this central area do not divide with the same rapidity as those towards the periphery of the cone, and so early lose their regular arrangement and show indications of maturation. This is indicated by the more rapid enlargement of the cells and the increase in thickness of their walls.

When the endosperm emerges into the loculus of the ovary a further change occurs in this median region of the cone. The intercellular spaces between those cells bordering the loculus enlarge and, as they continue to expand, those spaces lying more deeply in the tissue also become larger. Thus is formed an aerenchyma, into which the endosperm advances (Text-figure 16, A). As the ovary as a whole continues to expand, the aerenchyma penetrates further and further into this median region of the cone in the direction of the style.

Outside this median region, the parenchymatous cells, rich in tannin, retain their regular arrangement as they divide actively. Branched sclereids and 'stone' cells are differentiated, especially in the hypodermal region, while the vascular

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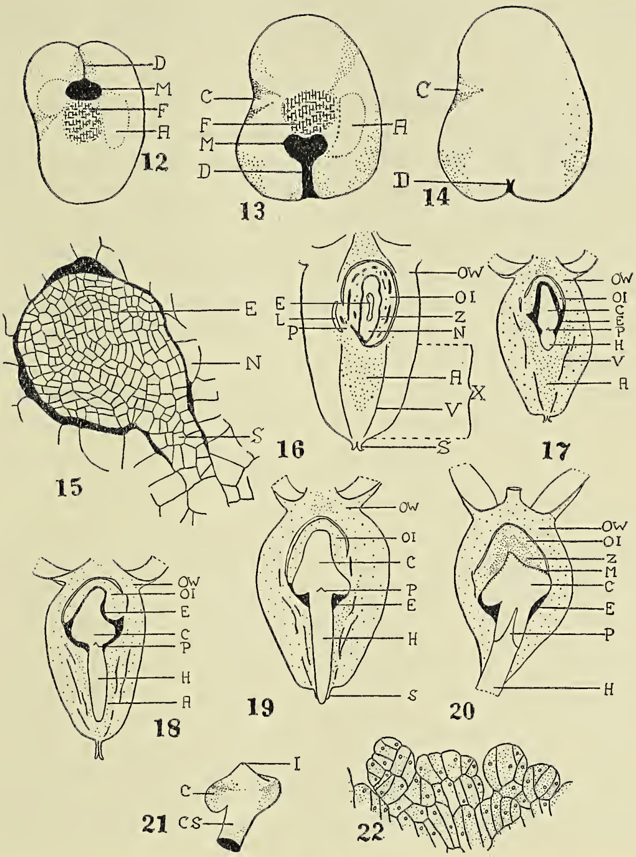
20.—A longitudinal section of a fruit of *Rhizophora mucronata* in which there has been an abnormal development of the inner zone of the integument. OW, ovary wall; OI, outer integument; Z, inner zone of the outer integument; M, meristematic zone of the cotyledons; E, endosperm; P, plumule; H, hypocotyl; C, cotyledons (× 0·8).

21.—The cotyledons removed from the fruit shown in Text-figure 20. C, head of the cotyledons; CS, cotyledonary sheath; I, indentation between the two cotyledons (× 0·8).

22.—A portion of the surface of a cotyledonary head showing the papillae (× 208).



Text-figs. 12-22.

12.—A developing seed prior to the emergence of the endosperm from the micropyle. F, funicle; A, position of contact of the aborted ovules; M, micropyle at which the face of the endosperm can be seen; D, slit in the integument (× 5).

13.—A developing seed of *Rhizophora mucronata* in which the endosperm is beginning to emerge. F, funicle; A, position of contact of aborted ovules; M, micropyle; D, widening slit in the integument; C, constriction caused by the placenta (× 4·5).

14.—The other side of the seed represented in Text-figure 13. D, the limit of the depression in the integument; C, constriction caused by the placenta (× 4·5).

15.—An embryo of a seed of *Rhizophora mucronata* such as is seen in Text-figure 9. N, endosperm; S, suspensor; E, meristematic cells of the embryo (× 112).

16.—A fruit of *Rhizophora mucronata* in which the endosperm is passing through the enlarged micropylar opening of the seed. OW, ovary wall; OI, outer integument; E, embryo; L, aborted loculus; P, placenta; N, endosperm; Z, inner zone of the outer integument; A, median region of the ovary wall; V, vascular strands; S, fused styles (× 5·5).

17.—An older fruit of *Rhizophora mucronata* showing the development of the embryo. OW, ovary wall; OI, outer integument; E, endosperm; C, cotyledons; P, plumule; H, hypocotyl; A, aerenchyma; V, vascular strands (× 0·8).

18.—An older fruit than that shown in Text-figure 17. OW, ovary wall; OI, outer integument; E, endosperm; C, cotyledons; P, plumule; H, hypocotyl; A, aerenchyma (× 0·8).

19.—A fruit from which the hypocotyl is emerging. OW, ovary wall; OI, outer integument; E, endosperm; C, cotyledons; P, plumule; H, hypocotyl; S, style (× 0·8).

strands increase in size and number. Thus this outer region is gradually modified so that later it is able to support the weight of the large embryo.

Development of the Embryo.

The invasion of the aerenchyma of the ovary wall by the endosperm causes a lysis of the invaded tissue, so that a fluid may actually lie between the advancing endosperm and the unaltered cells. Meanwhile the embryo retained within the developing seed shows an extensive growth of the cotyledons (C) which, by their enlargement, eventually push the hypocotyl (H) through the micropyle into the ovary wall (Text-figure 17). Then the hypocotyl, by its own elongation, passes through the enveloping endosperm into the aerenchyma of the ovary wall, which now occupies all the median region of the cone. The endosperm thus remains as a collar around the cotyledonary part of the embryo as noted by Haberlandt (1895).

The hypocotyl elongates into the aerenchyma by the resorption of the cells in advance of it (Text-figure 18). These cells are more or less uniform in size, but there is an increasing tendency for their elongation in a vertical direction. This is due to pressure exerted by the continued divisions of the cells outside this zone and by the enlargement of the seed.

This growth of the hypocotyl is accompanied by a slower but continued enlargement of the cotyledonary head at the expense of the endosperm (Text-figure 18, C). It will be noted that the growth of the cotyledonary head is differential, being governed by the nature of the surrounding cells. Where the advance is made into large thin-walled endospermic cells outside the micropyle, the greatest expansion of the head occurs. Inside the integument, the cells of the inner zone of that tissue, by the rate of their division, govern the degree of expansion of the head. In Text-figure 20 there has been an abnormal development of this inner zone (Z), so that the apex of the cotyledonary head is conical (Text-figure 21) instead of its usual shape. In such a cotyledonary head a minute depression at the apex marks the division between the two fused cotyledons.

The cotyledonary head when fully formed resembles a "Phrygian cap" (Karsten, 1891), and occupies the whole of the integument except for a single layer of endosperm. It conceals the plumule (P) at its base (Text-figure 19). By the time the cotyledonary head has so developed, the hypocotyl has pierced the ovary wall, by forcing its way between the two fused styles where the vascular strands converge (Text-figure 19, S). The pressure and resultant injury caused by this action are felt by a cap of loose spongy tissue which covers the meristem at the end of the hypocotyl. The elongation of the hypocotyl is accompanied by an increase in girth of that organ, which continues to maturity (Plate xv, fig. 1, A). Thus the aperture through which it emerged from the fruit is gradually widened by growth pressure.

The final stage in the development of the embryo is the elongation of the cotyledonary sheath (CS, Text-figure 24), in response to the plumule within it. The base of this sheath eventually passes through the aperture of the fruit (Plate xv, fig. 1, B) so that later the concealed plumule and hypocotyl can drop freely to the mud.

These phases in the growth of the embryo were outlined by Karsten, but no account is given by him of the cell structure of the ovary wall and the endosperm.

Structure of Mature Ovary Wall.

The now massive embryo is held in position by the extremely rigid wall of the fruit. At the periphery there is a zone of compact tannin-filled cells among which 'stone' cells and sclereids occur (especially the former). This grades into a spongy tissue, in which the spaces between the elongated parenchymatous cells are large. In spite of this, however, the area is rigid owing to the presence of many branched sclereids which project into these air spaces. The nearer the centre of the ovary wall the larger the spaces become and the greater the signs of pressure shown by the cells. Those few cells which yet remain of the original aerenchymatous zone of the wall show a pronounced elongation in a vertical direction, the air spaces between them being long and narrow. During this elongation some of the cells lose their rectangular form and taper almost to a point at one end while retaining their original width at the other. They contain an abundance of tannin and greater quantities of calcium oxalate than previously. It seems significant that the extension of the air space system of the ovary wall should continue as the embryo grows.

Nutrition of the Embryo.

The embryo, when it is initiated, receives its nutriment from the endosperm, which at that time is rapidly extending under the action of its peripheral meristematic layer. There is an intimate junction along an irregularly undulating line between this layer (M) and the integument (Z) (Text-figure 11). During and after the passage of the endosperm through the micropyle the embryo grows at its expense. As the cotyledons become larger and larger the endosperm within the integument seems gradually to lose its meristematic properties. The line of junction between the endosperm and the integument gradually straightens out and eventually the peripheral layer, which has ceased to divide, alone remains unresorbed. Its cells enlarge and form a sharply defined layer between the cotyledons and the integument (Text-figure 23, E). This is the layer which was noted by Haberlandt and compared by him, with regard to its appearance, with the epithelium of the embryos of grasses. This layer, the last remnant of the endosperm, has by now also lost its power to secrete enzymes for the digestion of the foods in the integument, so we find Haberlandt recording his failure to detect any secretion by this layer at all. But, failing to realize its endospermic nature, he wrote as follows: "Das Endosperm umhüllt nicht nur den in der Samenschale steckenden, kegelförmigen Theil des Cotyledonarkörpers sondern legt sich in Gestalt eines breiten Kragens auch an den Wulst und an den obersten Theil der Cotyledonarscheide an. Gegen die Samen-resp. Fruchtschale grenzt sich das Endosperm ringsum *ganz glatt* mit einer Zellschicht ab welche durch die Form ihrer Zellen, den reichen plasmatischen Inhalt und die Beschaffenheit ihrer verdickten Zellwände lebhaft an die 'Kleberschicht' des Gramineen-Endosperms zur Zeit der Keimung erinnert." This layer is, however, endospermic, but in the later stages of the development of the embryo its haustorial function is passed over to the cotyledons themselves.

The expansion of the cotyledons within the integument takes place by the division of a comparatively wide peripheral zone of densely protoplasmic cells. It becomes increasingly obvious that the divisions in these cells do not occur at an even rate. As a result the surface becomes uneven and appears to be 'warted' and papillate (Text-figure 22), as noted by Haberlandt. He found that these cells actively secrete enzymes, and there is no doubt that in the older embryos the function of obtaining food is passed from the endosperm, which is almost digested, to the cells of the cotyledons. But Haberlandt, with access to old embryos only, implies that the cotyledons alone are responsible throughout the life of the embryo. ("Die oberste Lage ist mit äusserst zahlreichen, ein-bis mehrzelligen Papillen und Wärzchen versehen, welche die absorbirende Oberfläche bedeutend vergrössern und die eigentlichen Saugorgane, die Haustorien des Keimlings verstellen.")

A section of an old seed (Text-figure 23) shows that the papillae of the cotyledons (C) do not lie in intimate contact with the single layer of the endosperm (E), but are separated from it by a nutritive fluid in which indistinguishable remnants of digested endospermic cells (R) may be seen. The endospermic layer lies close to what remains of the inner zone (Z) of the integument. The divisions of the cells of this zone have not kept pace with their resorption by the cotyledons, and therefore the width of the zone has been reduced to two or, in places, three cell layers. Behind this zone lie the mature cells of the rest of the integument, through which pass the conducting strands. Thus the embryo is nearer the source of food supply at maturity than at any other time.

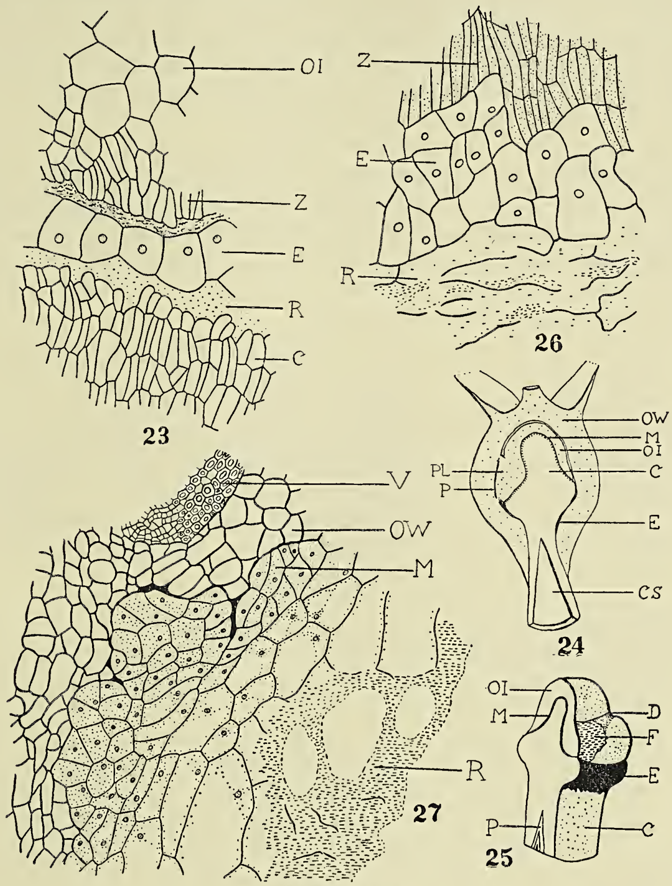
The growth of the large cotyledonary head exerts a definite pressure on the integument. This is shown by the cells of the inner zone, whose long axes become obliquely directed towards the embryo, instead of remaining at right angles to it, as they originally were.

Outside the seed, food is obtained from the ovary wall. This is passed to the embryo by the cells of the endospermic collar (E), which surround first the hypocotyl (H) and later the cotyledonary sheath (CS) (Text-figures 19, 24, 25). Haberlandt (1895) records that the endosperm merely lies in intimate contact with the ovary wall, but the present investigations show that shallow haustorial folds are formed. They are certainly not deep, not to be compared with those which occur, for example, in *Aegiceras majus*, but nevertheless seem to be quite definite. The divisions of the endosperm outside the seed are so rapid that several layers of equally dense cells are found at the periphery; thus the identity of the single meristematic layer is lost. These divisions result in the formation of haustorial folds at intervals right round the endospermic collar. Some longitudinal sections fail to show their presence, while in others they are quite distinct. The first indication of their formation is seen in a fruit in which the hypocotyl is elongating rapidly through the ovary wall (Text-figure 18). Their development continues as the embryo ages till by resorption they (M) come into close proximity to the vascular strands (V) of the ovary wall (Text-figure 27).

These folds are very shallow, due possibly to the degree of compactness of the cells invaded and the degree of concentration of food within them. Rather deeper folds were found in a fruit in which there had been an abnormal development of the inner zone of the integument (Text-figure 20). In that case, owing to the abnormality, folds were found penetrating the integument in the region of the micropyle as well as the ovary wall. An examination of Text-figure 26 shows that in the micropylar region (Z) the folds were deeper and narrower.

In the endospermic collar there is a gradual transition, as one would expect, from the actively dividing cells to those which are being resorbed in the vicinity of the embryo. The latter are large, with little cytoplasm, and stain but faintly with dyes (Text-figure 27). These are the 'bladdery' cells whose function Haberlandt suggests may be that of a water reservoir.

Thus in the nutrition of the large embryo, both the endosperm and the papillae of its cotyledons play a part.



Text-figs. 23-27.

23.—A detailed examination of a portion of an almost mature fruit. OI, outer integument; Z, inner zone of the integument; E, endosperm; R, resorbed endosperm; C, meristematic zone of the cotyledons (x 208).

24.—A fully mature fruit of *Rhizophora mucronata.* OW, ovary; OI, outer integument; C, cotyledons; M, meristematic zone of the cotyledons; PL, placenta and funicle; P, aborted loculus; CS, cotyledonary sheath to the plumule; E, endosperm (x 0·8).

25.—The seed and upper part of the embryo removed from a fruit such as that of Text-figure 24. OI, outer integument; M, meristematic zone of the cotyledons; F, funicle; D, depression in the seed left by the tightly drawn placenta which has been removed; E, last remnants of the endosperm; C, cotyledons; P, plumule (x 1·2).

26.—A detailed study of those tissues which surround the cotyledonary head at the micropylar end of the mature seed of Text-figure 20. Z, inner zone of the outer integument; E, last remnants of the endosperm; R, resorbed endosperm (x 208).

27.—A detailed study of the tissues outside the integument in a mature fruit. OW, ovary wall; V, vascular tissue of the ovary wall; M, meristematic zone of the endosperm; R, resorbed endosperm lying next to the cotyledonary sheath (x 112).

Mature Embryo.

The extraordinary size of the mature embryos of this genus is so well known as not to require any further mention (Plate xv, fig. 2, A). The vascular strands of the cotyledonary sheath snap and the plumule and hypocotyl fall to the ground (Plate xv, fig. 2, B). The hypocotyl is rigid enough to penetrate the mud (Kerner, 1894), in spite of the presence of aerenchyma, because of the development of a multitude of branched sclereids. The structures concerned in the aeration of this massive hypocotyl are discussed by Madeline Carson (1907).

Stored Foods.

In this discussion no mention has been made of the nature of the food concerned in the nutrition of the embryo. Throughout the development from the ovule in the flower to the shedding of the embryo from the fruit, the content of all parenchymatous cells of the integument stain very deeply with dyes. This is true to a less extent of many of the cells of the ovary wall and also of the embryo as it builds up its own food supply.

Microchemical tests (Haas and Hill, 1928) show the presence of great quantities of starch in the hypocotyl of the mature embryo. The abundant general occurrence of tannin is evident especially in the integument, placenta and cotyledons. The rôle played by tannin in the physiology of the mangroves is still a matter of dispute. Various hypotheses have been advanced (Bowman, 1917), but none of them are entirely satisfactory. It is thought that proteinaceous foods may be present also, their reaction masked by the presence of tannin. Bowman (1917) records dextrose in the seedlings of *Rhizophora Mangle.*

CERIOPS CANDOLLEANA Arn.

The developmental stages of *Ceriops Candolleana*, from the opening of the flower bud to the shedding of the embryo, are essentially the same as those seen in *Rhizophora mucronata.* However, there are minor differences which make a comparison interesting.

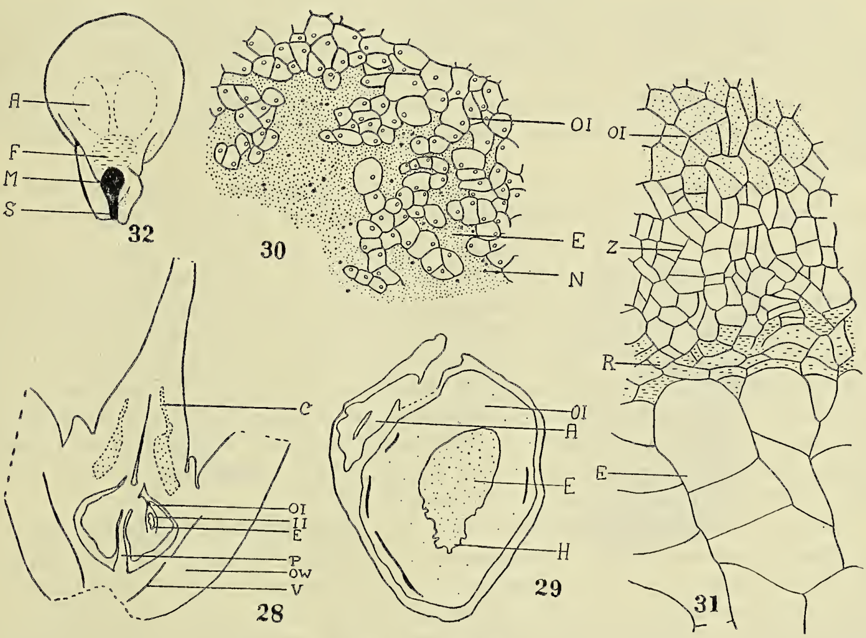
The general features of the flower are as well known as those of *Rhizophora,* and therefore require no further comment. However, it must be remembered throughout the discussion that the flower, and consequently the embryo arising from it, is much smaller than that of the genus already described. Karsten takes *Ceriops Candolleana* as his type genus with which he contrasts *Rhizophora.* His description of *Ceriops*, however, is no fuller than his account of *Rhizophora* already discussed.

The Gynoecium.

The wall of the ovary is composed of parenchymatous cells, among which 'stone' cells and sclereids are not developed. Another result of the smaller size of the flower is the absence of aerenchyma. This type of tissue does appear later, however, in association with the developing embryo. The parenchymatous tannin-filled cells of the ovary gradually merge into those of the style. These cells contrast sharply with a zone of clear cells lying beneath the hypodermis. This zone extends in the direction of the style, from an area in the ovary wall just above the loculus. At its upper end it is only one cell wide (Text-figure 28, C). On the inside of this zone lie the vascular strands. These enclose parenchymatous cells, also vertically arranged, but smaller in size than those nearer the periphery.

Development of the Ovule.

The ovary is trilocular or bilocular, each chamber holding two anatropous ovules. The structure and early development of each ovule is similar to that of *Rhizophora mucronata.* However, the inner integument does not appear to have retained its meristematic properties to the same extent and its rectangular cells stain but lightly with dyes such as aniline blue. The outline of the ovule is sinuous through the irregular growth of the cells of the outer integument, the greatest development taking place in the chalazal region, so that the embryo sac (E) seems to be placed well forward at the micropylar end of the ovule (Text-figure 28). The cellulose walls of the outer integumental cells are comparatively thicker than those in *Rhizophora.* The whole of this tissue, particularly the peripheral layers, stains deeply with dyes owing to the accumulation of tannin and foods in the cells. Thus the outer integument stands out sharply from the inner. Definite spiral thickenings are initiated in the vascular



Text-figs. 28-32.

28.—A longitudinal section of the ovary of an open flower of *Ceriops Candolleana.* OW, ovary wall; P, placenta; V, vascular tissue; OI, outer integument of the ovule; II, inner integument; E, embryo sac (x 11·5).

29.—A longitudinal section of an ovule of *Ceriops Candolleana* in which the embryo sac is enlarging. E, embryo sac; H, haustorial region of the embryo sac; OI, outer integument; A, aborted ovule (x 23).

30.—A detailed study of the haustorial region of the embryo sac shown in Text-figure 29. OI, outer integument; E, embryo sac; N, deeply staining granules (x 208).

31.—A detailed study of part of a seed in which the endosperm has been formed. OI. outer integument; Z, inner zone of the outer integument; R, resorbed tissue of the integument; E, endosperm (x 208).

32.—A seed of *Ceriops Candolleana* in which the endosperm is emerging through the micropylar opening. F, funicle; M, micropyle; S, slit in the integument; A, position of contact of aborted ovules (x 7).

zone, which extends right round the integument as in the ovule of *Rhizophora mucronata.*

After the resorption of the inner integument at the chalazal end of the ovule, the embryo sac (E), as it enlarges, develops haustorial projections (H) into the outer integument (Text-figure 29). Thus the embryo sac makes the greatest advance into that region of the integument which has shown the most extensive development. In Text-figure 30 the intrusion of the embryo sac (E) into the integument (OI) is shown in detail, also the deeply staining granules (N) which lie throughout the sac tending, at the periphery, to aggregate in groups.

By this time some of the ovules are aborted and eventually all are pushed to one side by the most vigorous, which occupies then all the space of the loculus. It is evident that the tissue of the ovary does not expand quickly enough to allow the development of more than one ovule from this stage onwards.

Post-fertilization Development.

When the ovule has enlarged to approximately three times its original size, it is fertilized and an endospermic tissue of large thin-walled cells is formed radiating out from the centre of the embryo sac. There is a gradual transition from those cells at the centre to the peripheral ones which are dividing. Thus there is no sharp definition of meristematic tissue as there is in *Rhizophora mucronata.*

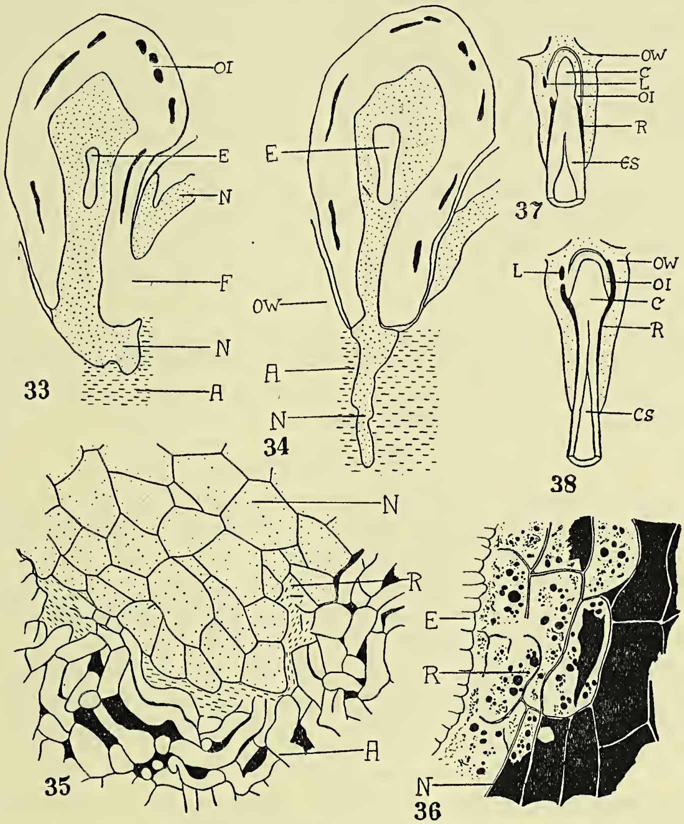
With the formation of the endosperm the integument is differentiated into two zones. The outer and larger zone, although not nearly as massive as that in *Rhizophora mucronata*, develops a comparatively extensive vascular system. The parenchymatous cells among these strands are rich in tannin and food material. The inner zone is comparatively narrow and composed of cells rather irregularly arranged (Text-figure 31, Z; cf. *Rhizophora mucronata*), but dividing at a rate equal to that of the intrusion of the endosperm (E) into them, so that this zone maintains a fairly constant diameter. The rate of resorption of the integument at first seems to be greatest at the chalazal end of the developing seed, where there may be quite a wide band filled with disintegration products between the endosperm and the tissue being invaded. Elsewhere the two tissues (Z and E) lie in direct contact (Text-figure 31).

As the integument increases in size the micropyle becomes longer but does not lose its tubular form. This passage is widened, however, by the divisions in the inner zone of the integument. This widening must be mechanical, as there is no evidence of haustorial action on the part of the endosperm as it passes down it. By the differential growth of the integument at the lower end of the micropyle a slit is formed as in *Rhizophora mucronata.* This slit (S) expands with the tubular micropyle (M) (Text-figure 32), till an aperture of considerable size is formed, through which the endosperm emerges. As it passes out into the loculus it increases in extent so rapidly that it folds back over the seed, sometimes completely enveloping the latter. At the same time its folds (N) enter the median region of the ovary wall (A) (Text-figures 33, 34) and to some extent the placenta in the funicular region (F).

Development of the Ovary Wall.

During this development changes have taken place in the ovary wall resulting in the formation of a cone of tissue between the loculus and the base of the style as in *Rhizophora mucronata.* This cone shows the same features as that of

Rhizophora, except for the lack of an extensive development of strengthening tissue. Thus in the median region an aerenchyma is formed, whose cells have lost their uniformity through strains put upon them as they matured, by the continued growth of the peripheral tissues. The advance of the face of the endosperm (N) into this tissue is shown in Text-figure 35.



Text-figs. 33-38.

33.—A longitudinal section through a seed of *Ceriops Candolleana* from which the endosperm is penetrating the ovary wall. OI, outer integument; E, embryo; N, endosperm; F, funicle; A, median region of the ovary wall which is aerenchymatous (× 11·5).

34.—A tangential section of a seed such as that shown in Text-figure 33. N, endosperm; E, embryo; OW, ovary wall; A, aerenchyma (× 11·5).

35.—A detailed study of the advancing face of the endosperm shown in Text-figure 33. N, endosperm; R, resorbed tissue of the ovary wall; A, aerenchyma (× 112).

36.—Endosperm in contact with the growing embryo as shown in Text-figure 33. E, embryo; R, endosperm being resorbed; N, endosperm (× 112).

37.—A mature fruit of *Ceriops Candolleana*. OW, ovary wall; OI, outer integument; C, cotyledonary head; L, aborted loculus filled with endosperm; R, remnants of endosperm and resorbed tissue of the ovary wall; CS, cotyledonary sheath (× 1·2).

38.—A very large mature fruit of *Ceriops Candolleana* showing a tendency towards lateral expansion of the cotyledonary head. OW, ovary wall; OI, outer integument; C, cotyledonary head; CS, cotyledonary sheath; L, aborted loculus filled with endosperm; R, resorbed tissue of the ovary wall and remnants of endosperm (× 1·2).

Development of the Embryo.

While the advance is made by the endosperm, growth of the embryo progresses as in *Rhizophora mucronata.* Around the embryo (E) is a resorption zone (R) in which food supplies are gradually exhausted (Text-figure 36). The food material in the endosperm (N) is undoubtedly the same as that in the integument, for the cells of both tissues give the same reaction to dyes. In fact, the only distinction between the two lies in the extreme difference in size of their cells.

This embryo differs from that of *Rhizophora mucronata* in one feature only, namely the shape of the cotyledonary head. In *Ceriops Candolleana* this head is in most cases cylindrical at maturity (Text-figure 37, C). In a few of the larger fruits a slight lateral expansion corresponding to the extensive lateral growth of the cotyledonary head in *Rhizophora* is shown (Text-figure 38, C). In *Rhizophora mucronata* the lateral expansion of the head resting against the ovary wall holds the heavy embryo firmly (Text-figure 24). In *Ceriops Candolleana,* however, there is generally no such support, and although irregularities developed on the surface of the hypocotyl may be of some aid, the embryo is readily dislodged.

The hypocotyl, which attained a length of approximately 11 cm. in the material available, is strengthened by an extensive development of collenchyma beneath the epidermis. The lumina of the epidermal cells are very much reduced and the whole is covered by a thick cuticle.

Nutrition of the Embryo.

Inside the integument the embryo is nourished by the aid of, first, the endosperm and, later, the papillae which are formed over the surface of the cotyledonary head as in *Rhizophora mucronata.*

Outside the seed, the endosperm undoubtedly obtains food from the wall of the ovary and placenta, although it does not produce haustorial processes into any region. It merely lies in contact with the food-bearing areas.

The writer wishes to express her sincerest thanks to Professor Osborn, University of Sydney, for suggestions and kindly criticism throughout the course of the work, and to Mr. R. E. Vallis for the collection of material.

SUMMARY.

1. The young ovule of *Rhizophora mucronata* consists of two massive integuments surrounding a nucellus and an embryo sac.

2. Before the bud opens the embryo sac resorbs the nucellus. It then, by intrusive folds, invades the inner integument, which increases in size in spite of this invasion.

3. Within the embryo sac lie the synergids, the egg nucleus and the endosperm nucleus. No antipodal cells are seen. Numerous deeply staining granules are found in the cytoplasm.

4. By the time the ovule has increased to approximately four times its original size the inner integument has been completely resorbed and fertilization has occurred. An endospermic tissue is then laid down in the embryo sac.

5. The outer integument is differentiated into two zones, an outer which contains the extensive vascular supply, and an inner in which the cells are regularly arranged and meristematic.

6. The endosperm expands by the division of a peripheral layer of comparatively small densely protoplasmic cells. The identity of this single meristematic

layer is lost, at the micropylar end of the endosperm, owing to the rapidity of its divisions as it advances into the loculus of the ovary.

7. The micropyle is at this period an aperture of considerable size owing to the pressure exerted on it and the adjoining slit in the integument by the dividing cells of the inner zone of the integument.

8. The advance of the endosperm is more rapid than its penetration into the ovary wall, so it falls back over the integument of the developing seed.

9. The embryo is well differentiated even before the endosperm leaves the micropyle. It was seen first as an undifferentiated mass of tissue attached by a suspensor to the endosperm. Later, two almost wholly fused cotyledons and a hypocotyl are differentiated.

10. During this development changes have occurred in the ovary wall, giving rise to an extensive cone of tissue between the loculus and the base of the style. Into the aerenchymatous median region of this cone the endosperm advances.

11. It is soon overtaken by the hypocotyl which is being forced downward by the growth of the cotyledons, which can no longer be wholly retained within the integument. The further advance of the hypocotyl is made by its own elongation, through the aerenchymatous zone, till it forces its way between the two fused styles.

12. During the elongation of the hypocotyl the cotyledonary head continues its expansion till at maturity it is shaped like a "Phrygian cap". The rate of this expansion is governed by the nature of the surrounding cells.

13. The final stage in the development of the embryo is the elongation of the cotyledonary sheath, which conceals the plumule. This extension continues till the base of the sheath emerges from the fruit.

14. During the development of the embryo, food is obtained inside the developing seed from the integument by the endosperm in the early stages, and later by the papillae and 'warts' of the cotyledons. Outside the seed the endosperm is reduced to a collar, first round the hypocotyl and later around the cotyledonary sheath. This endosperm penetrates the ovary wall by extremely shallow folds, and thus obtains food from it. At maturity the embryo is in close contact with the vascular tissue of both the ovary wall and integument.

15. The tissues of the ovary wall are differentiated so as to support the weight of the extremely large embryo and at the same time supply the oxygen required. When ready for dispersal the vascular strands of the cotyledonary sheath snap and the embryo falls into the mud, leaving the cotyledonary head behind.

16. In general, the development of *Ceriops Candolleana* is similar to that of *Rhizophora mucronata*.

17. The trilocular or bilocular ovary contains two ovules in each loculus. Each has an outer massive integument and a smaller inner integument surrounding a nucellus and an embryo sac.

18. The nucellus and inner integument are soon resorbed and the embryo sac encroaches on the massive outer integument. At the chalazal end, where the integument is widest, haustorial processes are developed.

19. After fertilization an endospermic tissue is formed in the embryo sac. This increases by the division of its cells, but there is no one meristematic layer as there is in *Rhizophora mucronata*.

20. The outer integument is differentiated into two zones, the inner one of which is not as sharply defined as it is in *Rhizophora*, nor are the cells as regularly arranged.

21. The divisions in the inner zone of the integument widen the micropyle but do not alter its tubular form. At the lower end an aperture of considerable size is formed, as the slit in the integument is gradually widened and thus increases the size of the opening. The endosperm without resorption advances down this enlarged passage and enters the loculus of the ovary.

22. The endosperm invades the median region of the enlarged ovary wall as in *Rhizophora mucronata* and also may enter the placenta at the upper end of the funicle.

23. The development of the smaller embryo is similar to that of *Rhizophora mucronata*. Its cotyledonary head is, however, cylindrical in shape.

24. In the nutrition of this smaller embryo endospermic folds are not formed, and for its support less strengthening tissue is differentiated in the ovary wall.

EXPLANATION OF PLATE XV.

Fig. 1.—Developing embryos of *Rhizophora mucronata*. A, a fruit in which the hypocotyl is elongating downward outside the ovary wall; B, a fruit from which the base of the cotyledonary sheath at the top of the hypocotyl has emerged; C, a fruit in which two embryos are maturing (× 0·43).

Fig. 2.—Developing embryos of *Rhizophora mucronata*. A, a mature embryo; B, the embryo released from the fruit; C, the growing embryo (× 0·3).

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